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IODINE INTAKE ON THE THYREO-
GLOBULIN CONTENT OF THE
THYROID GLAND

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

August, 1933

By
MILDRED ELINOR JONES

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THE EFFECT OF VARYING LEVELS OF IODINE INTAKE ON THE THYREOGLOBULIN CONTENT OF THE THYROID GLAND¹

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Most investigators are agreed that inorganic iodine, fed or injected into an animal, in adequate amounts, for a sufficient length of time, increases the iodine content of the thyroid gland. This has been reported by Van Dyke (1), Krauss and Monroe (2), Evvard (3), Boruttau (4), Marine (5), Wheeler (6), Charvat (7), McClendon (8), and others, as a result of feeding KI to animals of various species. Contrary to these results Klein, Pfeiffer and Hermann (9), and Pfeiffer (10) did not find an increased iodine storage after KI administration to dogs. This discrepancy might be explained by differences of dosage and length of time of administration. Gray and Rabinovitch (11) in trying to correlate the degree of histological change with the size of the dose of KI, administered dosages of 0.0001, 0.001, 0.01, 0.05, and 0.1 gram per guinea pig, daily for fifteen to twenty days. The first dosage was ineffective, the second increased mitosis without otherwise producing an "active gland," and the last three caused histological changes typical of an "active gland" as well as a marked proliferation of epithelium. This mitosis was not proportional to the size of the doses. Gray, Haven and Loeb (12) found that feeding of KI produced no effect on the cells for thirty days, after which time the epithelium became lower and the acini enlarged. Van Dyke reported that when he fed one gram of KI daily to dogs for seventeen days, the total iodine content was increased but the ratio of cell iodine to total iodine was unchanged, whereas, when he injected 50 mgm. intravenously, if he removed the glands within sixty minutes, the total iodine was again high but the ratio of cell iodine to total iodine was very low. From the findings of these workers it would appear that Pfeiffer's dosage of only 3 mgm. daily and his time of administration of seven days were inadequate.

Although there has been considerable work done upon the histology of the gland and the storage of iodine, very little work has been done to determine the nature of the iodine compound stored. Marine and Rogoff

¹ This work was aided by the Biological Research Fund of the Rockefeller Foundation.

(14), after removing one thyroid from a number of dogs, injected 50 mgm. of KI intravenously, and then removed the second gland at intervals varying from a few seconds to thirty hours. The glands were compared for histological changes, iodine storage, and the activity of the substance stored as determined by the Gudernatsch test. Histological changes of an involutionary type were present in twenty hours. The storage of iodine was almost instantaneous whereas the elaboration of the hormone required a longer time, only a small fraction of the iodine taken up in a few seconds having been transformed into a specific hormone at the end of thirty hours. However, a slight difference in the activity between these glands and the controls was apparent in eight hours, and became more pronounced at the end of twenty hours. There was a great variation among dogs in the rate of formation of the active substance. This present investigation was carried out in order to determine a little more specifically the nature of the stored compound, and to see what effect large and small doses of KI would have upon the quantity stored.

PROCEDURE. Since the method employed required about 10 grams of thyroid material, dogs, because of the size of their thyroids, were chosen as the most suitable experimental animals. To obtain this quantity, the right glands of ten dogs were compositely analyzed as the control and later, after iodine administration, the left glands were similarly analyzed. This procedure was adopted rather than using both glands from two series of five dogs each, where one series would serve as the control and the other for the experiment, because previous investigations had shown that the variation of the thyroid iodine content between different dogs was very large, whereas that between the two lobes of the same dog was small. The value found for the average difference between the two lobes of the same dog in a study of ten was 0.09 mgm. per gram of fresh gland, which is of the same order of magnitude as the value of 0.0055 mgm. per gram found by Rahe, Rogers, Fawcett and Beebe (15) for seven dogs, and that of 0.0156 mgm. per gram found by Watts (16) for ten dogs. In contrast to these slight differences are the variations between the different series of ten or more dogs, where it was found in twelve such studies that the maximal and minimal iodine contents were 1.138 mgm. per gram and 0.222 mgm. per gram, respectively. In spite of these large discrepancies in total iodine, the per cent precipitable by alcohol was always within the range of 94.6 per cent to 99.9 per cent. Accordingly then, after the right thyroid had been removed from six series of ten dogs each the first two series were fed 0.1 gram of KI per kilo. daily for six weeks, the next two 0.02 gram per kilo., and the last were kept as controls to see whether the removal of one gland led to a compensatory increase in the iodine content of the remaining gland. The low dose was that found by Gray and Rabinovitch (11) to produce minimal histological changes in the guinea pig thyroid, and the

high one was half of their maximal, as larger quantities proved too toxic to be tolerated by some dogs.

The method for extracting the iodine from the glands was that devised by Barnes (17) using one volume of 0.1 N sodium acetate per volume of glands. A preliminary investigation of dog thyroids had shown that from 97 per cent to 99 per cent of the total iodine could be removed in five extractions. The glands were, therefore, thoroughly ground in a mortar and extracted five times during a period of eight days. The iodine content of the extract was determined on duplicate 5 cc. samples by Kendall's (18) method, and the iodine in the gland residue was determined by the same method. It was then possible to calculate the average milligrams of iodine per gram of fresh gland. Barnes and Jones (19) had found that by using three volumes of 95 per cent alcohol, they could precipitate thyreoglobulin and possibly other related heavy molecules, leaving any lighter iodine-containing molecules such as thyroxin in solution. This procedure was applied to duplicate 5 cc. samples, after which they were centrifuged and the iodine content of the precipitate determined by Kendall's method.

Some of the extract was tested for biological activity by means of the Feather Germ test described by Juhn and Barnes (20).

RESULTS. Owing to the loss of one or more dogs through snuffles, or the occurrence of an infected gland which had to be discarded, there were fewer dogs used in the analysis of the second gland than of the first in all groups except one. For this reason the weights are given in terms of average weight per gram of gland. The experiment for the groups marked A lasted six weeks while that of those marked B was terminated at the end of four weeks. The total milligrams of iodine per gram of gland were calculated from the total iodine extracted and the weight of the glands used. The extractions were always 98.0 per cent to 99.9 per cent complete. The results of the chemical analysis are shown in table 1.

The loss of iodine observed in control series A can not be explained; the gain in control series B is insignificant when compared to that of the KI fed groups. The amount of iodine stored was independent of the length of time of iodine administration, and of the dosages used but seemed to bear an inverse relation to the original iodine content of the gland. Most of the dogs showed no ill effects from the KI and were in good condition as judged by a study of body weights. There was a small loss of weight in some and a gain in others, but this was apparently not due to the KI administered for the same variations occurred in the control animals.

As judged from the Feather Germ test, the biological activity per unit of the iodine contained in the thyroid gland is about the same after iodine administration as before. In a series of twelve birds injected with control extract in doses containing from 0.386 to 0.084 mgm. of iodine, positive reactions were obtained for doses as low as 0.168 mgm. of iodine.

Thyroid extract after KI feeding, injected into six birds in doses from 0.706 to 0.176 mgm. of iodine caused pigment deposition in the feathers for all doses. Since the two series were injected at different times, to rule out any seasonal variation in the reactivity of the birds, thyroxin, the activity of which had been compared for all seasons, was injected into a similar series. This test while not absolutely quantitative showed the extract to be of the same order of activity per unit of iodine after iodine administration as before.

This work shows that KI in doses of 0.02 gram per kilo. daily for four weeks will produce as great storage of iodine as doses of 0.1 gram per kilo daily for six weeks. A certain minimal dose seems necessary, above which larger doses are superfluous. The Feather Germ test showed that this

TABLE 1

Showing the per cent of iodine increase and the per cent of iodine precipitable by 95 per cent alcohol

KI DOSAGE	NUMBER OF DOGS USED		AVERAGE WEIGHT PER GRAM GLAND		MG. OF IODINE PER GRAM GLAND		PER CENT OF IODINE PRECIPITABLE BY 95 PER CENT ALCOHOL		PER CENT OF IODINE INCREASE
	Right	Left	Right	Left	Right	Left	Right	Left	
A. Control.....	10	9	1.240	1.411	0.836	0.558	96.6	99.4	-33.6
B. Control.....	10	10	0.922	0.940	1.155	1.272	98.4	97.8	10.13
A. 0.02 gm. KI.....	10	9	1.300	1.444	0.623	2.010	95.7	95.6	248.3
B. 0.02 gm. KI.....	9	7	1.333	1.230	0.660	2.046	96.3	94.9	210.0
A. 0.1 gm. KI.....	10	9	1.348	1.388	0.526	1.837	94.6	99.3	249.22
B. 0.1 gm. KI.....	10	8	0.920	1.012	1.085	2.668	99.9	95.8	146.8

increase in iodine was accompanied by an increase in biological activity, and the chemical analysis showed that the iodine was in a form precipitable by 95 per cent alcohol. While either thyroxin or thyreoglobulin might give a positive Feather Germ reaction, this could not have been thyroxin, since a thyroxin solution of equivalent iodine content added to thyroid extract before precipitation does not lead to any increase in the iodine of the precipitate. This points strongly to the fact that thyreoglobulin is the product elaborated and stored by the gland.

SUMMARY

1. Iodine in the form of KI fed to dogs produced an increase of 146.8 per cent to 249.22 per cent in thyroid iodine, 94 per cent to 99.9 per cent of which was in a form precipitable by 95 per cent alcohol, and therefore, probably thyreoglobulin.

2. Dosages of 0.02 gram per kilo. daily for four weeks were as effective in producing iodine storage as those of 0.1 gram daily for six weeks.

3. The biological activity per unit of iodine in the thyroid gland as found by the Feather Germ test is of the same order of magnitude after as before iodine administration.

4. The evidence is strongly in favor of an increased formation and storage of thyreoglobulin during KI administration, under the conditions of this experiment.

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